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Molecular analysis of Pericentrin gene (*PCNT*) in a series of 24 Seckel/ MOPD II families

M. Willems¹, D. Geneviève¹, G. Borck¹, C. Baumann², G. Baujat¹, E. Bieth³, P. Edery⁴, C. Farra⁵, M. Gerard², D. Héron⁶, B. Leheup⁷, M. Le Merrer¹, S. Lyonnet¹, D. Martin-Coignard⁸, M. Mathieu⁹, C. Thauvin-Robinet¹⁰, A. Verloes², L. Colleaux¹, A. Munnich¹, V. Cormier-Daire^{1*}

1. Université Paris Descartes, INSERM U781, Department of Genetics, Hôpital Necker, Paris, France
2. Department of Genetics, Hôpital Robert Debré, Paris, France
3. Department of Genetics, Hôpital Purpan, Toulouse, France
4. Hospices Civils de Lyon, Groupement Hospitalier Est, Service de Cytogénétique Constitutionnelle, Bron cedex F-69677 et Université Lyon 1, Lyon, F-69003
5. Department of Pathology and Laboratory Medicine American University of Beirut Medical Center, Beyrouth, Lebanon
6. Department of Genetics, Hôpital de la Pitié-Salpêtrière, Paris, France
7. Department of Genetics, CHU de Nancy, Nancy-Université, Nancy, France
8. Department of Genetics, Hôpital du Mans, France
9. Department of Pediatrics, CHU d'Amiens, France
10. Centre de Référence Anomalies du Développement et Syndromes Malformatifs Grand Est, Centre de Génétique, Hôpital d'Enfants, CHU Dijon, France

*corresponding author : valerie.cormier-daire@inserm.fr

ABSTRACT

Microcephalic osteodysplastic primordial dwarfism type II (MOPD II, MIM 210720) and Seckel syndrome (SCKL, MIM 210600) belong to the primordial dwarfism group characterized by intrauterine growth retardation, severe proportionate short stature and marked microcephaly. MOPD II is distinct from SCKL by more severe growth retardation, radiological abnormalities and absent or mild mental retardation. Seckel syndrome is associated with defective ATR-dependent DNA damage signalling.

In 2008, loss-of-function mutations in the pericentrin gene (*PCNT*) have been identified in 28 patients, including 3 SCKL and 25 MOPDII cases [6, 7]. This gene encodes a centrosomal protein which plays a key role in the organization of mitotic spindles.

The aim of our study was to analyze *PCNT* in a large series of SCKL-MOPD II cases to further define the clinical spectrum associated with *PCNT* mutations. Among eighteen consanguineous families (13 SCKL and 5 MOPDII) and 6 isolated cases (3 SCKL and 3 MOPD II), we identified thirteen distinct mutations in 5/16 SCKL and 8/8 MOPDII including five stop mutations, five frameshift mutations, two splice site mutations and one apparent missense mutation affecting the last base of exon 19. Moreover, we demonstrated that this latter mutation leads to an abnormal splicing with a predicted premature termination of translation. The clinical analysis of the 5 SCKL cases with *PCNT* mutations showed that they all presented minor skeletal changes and clinical features compatible with MOPDII diagnosis. We therefore conclude that, despite variable severity, MOPDII is a genetically homogeneous condition due to loss-of function of pericentrin.

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KEY WORDS

Seckel syndrome

MOPDII

Skeletal manifestations

PCNT

INTRODUCTION

Among the primordial dwarfisms, microcephalic osteodysplastic primordial dwarfism type II (MOPD II, MIM 210720) and Seckel syndrome (SCKL, MIM 210600) are both characterized by intrauterine growth retardation, severe proportionate short stature and microcephaly [1, 2]. MOPDII is distinct from SCKL by the severity of the growth retardation, the presence of skeleton abnormalities and the mild/ absent mental retardation [3]. SCKL is a genetically heterogeneous condition associated with defective ATR-dependent DNA damage signalling [4]. The only reported genetic defect so far is a hypomorphic mutation in the *ATR* gene (*Sckl1*, 3q22.1-q24) [5]. In 2008, mutations in the pericentrin gene (*PCNT*) have been identified in 28 patients, including 3 patients with SCKL [6] and 25 with MOPDII [7]. This gene encodes a centrosomal protein, which acts both at structural and regulatory levels. First, pericentrin recruits several structural centrosomal proteins, particularly gamma tubulin ring complex which initiates microtubular nucleation and spindle organization [8, 9, 10, 11]. Second, it plays a role in cell cycle regulation through its interaction with the ATR pathway [6]. All mutations identified so far lead to premature translation termination and are responsible for pericentrin loss of function as demonstrated in *PCNT*-mutated cell lines issued from patients with SCKL or MOPDII.

To further define the clinical spectrum of patients with *PCNT* mutations, we analyzed *PCNT* in 24 families diagnosed either with SCKL or with MOPD II, including 18 consanguineous families and 6 cases from unrelated parents.

PATIENTS AND METHODS

Patients

Criteria for inclusion in the study were:

- Intrauterine and postnatal growth retardation with birth weight < -2 SD and postnatal height < -4 SD
- Microcephaly with an occipitofrontal circumference (OFC) < -4 SD
- Diagnosis of MOPDII or SCKL made by a clinical geneticist.

Diagnostic assessment was performed for all patients by their clinicians (Table 1). Seven of these families were previously clinically reported, by Faivre et al [12] (families 1,3,7, 8, 11 and 12) and Verloes et al [13] (family 19). Written informed consent was obtained from all subjects included in this study.

Microsatellites marker analysis

In all consanguineous families, microsatellites analysis of the *PCNT* locus was first performed and *PCNT* was sequenced only in compatible cases. The *PCNT* sequence analysis was performed in all cases from unrelated parents. Blood samples were obtained with informed consent from affected children, parents and unaffected siblings. Genomic DNA was extracted using Nucleospin® Blood XL kit (Macherey-Nagel). We established lymphoblastoid cell lines by EBV transformation and we performed a primary skin fibroblasts culture. Genotyping was performed using 4 flanking (D21S1903, D21S1897, D21SpolyATT, D21S1446) and one intragenic-*PCNT* (*PCNT*-IG) microsatellite markers in all consanguineous families and non-consanguineous families with at least two siblings.

Mutation analysis

PCNT exon and flanking intron sequences were amplified from patient DNA by PCR using 49 couples of primers designed with the Primer 3 software (Sequence of primers available on request). Sequencing reactions were performed on both strands using the BigDye Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems, Foster City, California) according to the manufacturer's instructions.

Functional consequences of c.3840G>C (p.Q1280H) mutation

RNA was extracted from cultured fibroblasts and cDNA was synthesized using primers designed to overlap 2 consecutive exons (Sequence of primers: JUNCTION17/18-5': CCTGTCCCACAGCGAAAGAG; JUNCTION18/19-5': ACAGCTCCGGCGCTGGAG; JUNCTION20/21-3': GGGTACCCTGAGTCTTGTGCAGC). RT-PCR products were expected to span exons 18 to 20 and exons 19 to 20. GAPDH expression was used as positive controls.

RESULTS

Genotyping analysis showed that 9/18 consanguineous families (4/13 SCKL and 5/5 MOPDII) were compatible with linkage to the *PCNT* locus.

The *PCNT* sequence analysis performed in these 9 consanguineous families and in 6 additional cases (from unrelated parents) allowed the identification of 13 distinct mutations in 13 patients (Table 2). All but one mutation were homozygous and cosegregate with the disease. In one patient (case 22), a single heterozygous mutation, inherited from the father, was detected. The 13 mutations were located throughout the gene and among them, five were nonsense mutations (exons 11, 15, 17, 28 and 34), five were frameshift mutations (exons 14, 16, 28A, 30), two were splice site mutations (intron 18 and intron 40) and one was an apparent missense mutation affecting the last base of exon 19 (c.3840G>C, p.Q1280H). None

of the mutations were identified in 400 control chromosomes. The c.3840G>C mutation was predicted to alter splicing. This was further confirmed by sequence analysis of RT-PCR products which demonstrated exon 19 skipping, predictive of a premature termination of translation (p.P1204GfsX11).

Among the 13 patients, 5 were clinically diagnosed as SCKL (patients 12-16) and 8 were diagnosed as MOPDII (patients 17-24, Table 1). The identification of *PCNT* mutations in 5 SCKL and 8 MOPDII patients prompted us to re-analyze the clinical features of all the patients with *PCNT* mutations and compare them to SCKL patients without mutation. We first observed that the five SCKL patients with *PCNT* mutations (families 12-16) presented a more severe growth retardation than the SCKL patients without *PCNT* mutation (-6 to -8 SD versus -4 to -5 SD), but less severe than the MOPDII patients with *PCNT* mutation (-7 to -13 SD). For two of them, the adult height is 120 cm and 140 cm respectively (Figure 1, from Hall JG). These patients presented also skeletal anomalies including gracile long bones, metaphyseal flaring, carpal condensation, and moderate hip dysplasia (Figures 2, 3, 4). These anomalies were not present during the first year of life, became more pronounced with time but were often less severe than those classically described in MOPDII patients. Importantly, we did not observe similar skeletal anomalies in 6 patients without *PCNT* mutation and with skeletal survey available. Finally, these patients have either normal intelligence or mild mental retardation. No difference was observed with respect to age of walking (normal to slightly delayed) and developmental course. Early developmental milestones were considered as normal with excellent social skills. Learning disability was noted after the age of 5 years. None of the 4 adult patients can live independently and they all perform “adapted work”.

Other features suggestive of MOPDII diagnosis were present in the clinically diagnosed SCKL patients with *PCNT* mutations including truncal obesity and death at 20 years of age of rupture of CNS vessels (patient 15), initial feeding difficulties and development of typical pigmentation anomalies with time (patients 13 and 14), polycystic ovaries (patient 14), subglottic stenosis (patient 13), microdontia (patient 12), high-pitched voice, stridor and upper respiratory tract infections (patient 16).

Finally, facial features were highly suggestive of MOPD diagnosis for 11 patients with *PCNT* mutations including a broad nose with hypoplastic tip, thin alae nasi, with columella lying below the alae nasi, long midface, prominent cheeks, small jaw and large eyes in the youngest children. Facial features were also changing with time, variable with the ethnic origin (patient 18) and less characteristic in the eldest patient of our series (patient 15). By contrast facial features of the patients with no *PCNT* mutation (1-11) were quite variable

(Figure S1) mainly dominated by the microcephaly with receding or short forehead and relatively large ears.

DISCUSSION

We report here the identification of 13 distinct mutations in 8 MOPDII and 5 SCKL patients. As previously reported by Griffith [6] and Rauch [7], mutations are distributed throughout the gene. We did not find any recurrent mutations in our series. However, the c.3109G>T mutation (exon 15) was previously reported by Rauch *et al* in a patient also originating from Turkey (patient 1). In one patient (patient 22), one mutation only was detected by direct sequencing but unfortunately RNA was not available. This might be due to the limit of our screening and a partial deletion of *PCNT* gene cannot be excluded. Our study provides also the first example of a “missense” mutation (c.3840G>C) but we demonstrated that this mutation impairs exon 19 splicing, leading to premature termination of translation. We conclude, as previously suggested, that all identified mutations are loss of function mutations.

We identified *PCNT* mutations in all MOPDII cases, confirming the genetic homogeneity of this disorder. Moreover, the retrospective analysis of the 5 SCKL patients with *PCNT* mutation also suggests that they all belong to the MOPDII spectrum. However, they were diagnosed as SCKL, based on the absence of severe skeletal manifestations and on their final stature >110 cm, which usually excludes the diagnosis of MOPDII [3]. Our study also supports that SCKL spectrum is heterogeneous and suffers from variable definition in the literature and from clinicians in practice. Indeed, Seckel syndrome has often been used as a generic term used for primordial dwarfism, without more specific diagnosis. Recently, D'Angelo and Di Bartolomeo reported two cases of SCKL with intracranial anomalies, suggestive of MOPDII diagnosis [16, 17]. Similarly, SCKL patients with bone dysplasia suggestive of MOPDII have been reported [18, 19]. From our study, we suggest that MOPDII spectrum is wider than previously defined. However, in all patients with *PCNT* mutations we have consistently observed 1) distinct facial features 2) growth retardation <-5SD and microcephaly <-4SD, 3) mild to absent mental retardation 4) skeletal manifestations including hip dysplasia ranging from short femoral neck to severe coxa vara; carpal condensation, and gracile long bones with metaphyseal flaring. Other suggestive features occasionally observed included 1) vascular anomalies and cutis marmorata 2) high pitched voice 3) microdontia, 4) hyperinsulinism, 5) subglottic stenosis, 6) pigmentation anomalies with areas of hypo- and hyperpigmentation.

We also observed in two patients with *PCNT* mutation a liver involvement varying in severity from cytolysis to cirrhosis, with the same histological features than those described in the literature for patients with MOPDI diagnosis, consisting in ductular cholestasis, inflammatory infiltrate, and giant multinucleate hepatocytes. Although these findings are not specific, they may suggest the existence of biliar epithelium anomalies in MOPDII spectrum [20].

While this study further demonstrates that MOPDII is caused by *PCNT* mutations, the pathogenic mechanisms underlying the clinical features observed in these patients remain unclear [21]. First, microcephaly could be related to structural centrosomal abnormalities similar to those observed in primary microcephaly [22]. Second, defect in ATR-dependent DNA damage signalling has been demonstrated in other conditions characterized by short stature and microcephaly and may thereby also account for short stature observed in MOPDII cases [23, 24]. Other specific clinical features like vessels anomalies, generalized bone dysplasia and hepatitis remain unexplained so far, since they have not been described in patients with ATR mutations or other centrosomal genes such as *ASPM*.

Finally, O'Driscoll and collaborators suggested that the ATR signalling pathway was unusually sensitive to haploinsufficiency and established a correlation between ATR-pathway dysfunction and growth retardation [23]. Similarly, Rauch et al reported a significant reduction of the mean height of heterozygous MOPDII parents [7]. We did not observe such a reduction in the mean of parental heights (ranging from -1 to +2 SD) but ethnical variability of growth charts may interfere with parental height analysis.

In conclusion, we identified 13 *PCNT* loss of function mutations in 13 patients who all presented diagnostic criteria for MOPDII. However, we observed a wider variability in the severity of the short stature and skeletal manifestations than previously admitted, modifying the MOPDII clinical spectrum. The distinction between SCKL and MOPDII appears to be crucial for the appropriate management, keeping in mind the risk of vascular anomalies in MOPDII.

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Legends to figures

Figure 1: Comparison of growth curves of patients from our cohort with those of patients from MOPD II cohort reported by Hall JG et al (from Hall JG, 2004)

Figure 2: Comparison of Hip X-rays in patients with PCNT mutations.
Note the hip dislocation and coxa vara more pronounced in MOPD II patients

Figure 3: Comparison of lower limb X-rays in patients with PCNT mutations.
Note the gracile long bones in both groups.

Figure 4: Comparison of hand X-rays in patients with PCNT mutations
Note the carpal fusion, the clinodactyly and the delayed bone age.

Figure S1. Facial features in two sisters with no *PCNT* mutation (A and B, family 7) and two patients with *PCNT* mutation (C1 and C2, Patient 14; D1 and D2, Patient 17).
Note in patient with *PCNT* mutations the characteristic nose with hypoplastic alae nasi, prominent cheeks and small jaw while in the two patients with no *PCNT* mutation the facial features are marked by the severe microcephaly with large nose and large ears.

Family	Ethnic origin/ Gender	CS /Coefficient f	Birth : WOG/ Weight(g)/Heigh t(cm)/HC(cm)	Postnatal growth : Weight /Height/ HC (SDS)	Mental retardati on	Other clinical features	Radiological features
1 /SCKL* (case 4[12])	Algeria F	Y f=1/64	41/2160/45/32	-4/-4/-4	Mild	Café au lait spots. VSD. Pyelic bifidity. Osteosarcoma	Mild bowing of the femora
2/SCKL	NA M	Y	NA	NA	NA		NA
3/ SCKL* (case 3[12])	Mali M	Y f=1/8	FT/2000/40/24	-4/-5 ,5/-10	Severe	Hypertonia. Ichtyosis. Abnormal gyration pattern	Slight platyspondyly
4/SCKL	France M	Y f=1/16	NA	NA	NA	NA	NA
5/SCKL	Algeria F/F	Y	NA	NA	NA	NA	NA
6/SCKL	Algeria M	Y f=1/32	FT/3300/47/30,5	-4/-4/-6	Mild	Hip dislocation. Chromosomal breakage	Normal
7/SCKL* (case 2[12])	Morocco F/F	Y f=1/16	FT/1400/40/28 FT/NA/40/NA		Mild/ N	Delayed puberty. Cataract Catarct	Thoracolumbar scoliosis
8/SCKL* (case 5[12])	Algeria M/M/M	Y f=1/16	FT/2040/43,5/30 FT/2140//42/NA FT/2300/NA/NA	NA/-5/NA NA/-5/NA NA/-4/NA	Mild	Cryptorchidia.Diabetes mellitus. Craniosynostosis	Scoliosis. Thick long bones.
9/SCKL	Lebanon F	Y NA	NA	NA	NA	NA	NA
10/SCKL	France F/F	N	FT/2030/41/NA NA/NA/NA/NA	-3.5/-7/-6 -5/-8/-7.5	N/ Mild	Pyelic duplicity Scoliosis	NA Severe scoliosis
11/SCKL* (case 6[12])	France M/F	N	NA	NA	NA	NA/-4/-8 NA/-4/-6	NA
12/ SCKL* (case 1[12])	Morocco F/NA (TOP)	Y f=1/16	37/1340/38/28	-8/-8/-8	Mild	Microdontia. Pyelic ectasia	Thick diaphyseal cortex. Carpal fusion. Gracile long bones. Brachymesophalangia
13/SCKL	Morocco F	Y f=1/16	32/950/34/25,5	-5/-8/-6.6	N	Café au lait spots, areas of depigmentation Hepatic cytolysis. Subglottic stenosis Recurrent upper respiratory tract infections	Coxa vara
14/SCKL	Pakistan F	Y f=1/16	FT/1650/42/30	-3.5/-7/-5	N	Café au lait spots, area of depigmentation. Polycystic ovaries. Chromosomal breakage	Gracile long bones. Short femoral neck. High vertebral bodies. Carpal fusion. Overtubulated and thick diaphyseal cortex.
15/SCKL	France M	N	FT/1720/40/27,3	-2.7/-6/-8	Mild	Horseshoe kidney. Clinodactyly of fifth finger. Rupture of CNS vessels leading to death (20 years)	High vertebral bodies. Thick diaphyseal cortex. Short femoral neck
16/SCKL	Lebanon M	Y F=1/16	NA/800/30/NA	NA/-9/-13	Y	Sparse scalp hair. Receding forehead. Prominent curved nose, Micrognathia. Low set ears. Clinodactyly. High-pitched voice. Stridor. Upper respiratory tract infections	Brain MRI : minimal bilateral ill- defined areas of hypersignal intensity in white matter (terminal zone of myelination) Skeletal X rays: NA

17/ MOPDII	Turkey M	Y f=1/16	36/1290/35/27	-6/-9/-7	Mild	Poor sucking.Vomiting. Hyperlaxity. Horseshoe kidney.Hypertonia. Subglottic stenosis. Recurrent upper respiratory tract infections. Micropenis High squeaky, nasal voice	Delayed ossification. Coxa vara
18/ MOPDII	Sri Lanka/ M	Y f=1/16	NA	NA/-12/-12	NA	Poikiloderma. Atrial septal defect.	Hypoplastic distal phalanges. Carpal fusion. Hip dislocation. Overtubulated and thick diaphyseal cortex
19/ MOPDII *[13]	Morocco F/M	Y f=1/16	37 /770/30/24 39/1190/33/27,6	-7/-11/-12 -9/-10/-10	Severe/ severe	Hypertonia. Micropenis.	Radial, ulnar, and femoral metaphyseal flaring.
20/ MOPDII	Algeria M	Y f=1/16	FT/NA/30/NA	-10/-13/-10	Mild	Café au lait spots. Moya- moya disease complicated with rupture of CNS vessels.	Coxa vara. Carpal fusion
21/ MOPDII	Italy M	N	31/585/31/23,5	-8/-12/-12	N	Micropenis. Café au lait spots . Livedo reticularis. Cirrhosis. High squeaky, nasal voice	Coxa vara
22/ MOPDII	France M	N	37/1400/40/29,5	-9/-7/-6	N	Anemia. Body asymmetry Radial head dislocation	NA
23/ MOPDII	Morocco F	N	37/870/33,5/24	-6/-7/-9	N	High squeaky voice. Café- au-lait spots	Coxa vara. Short femoral neck. Delayed bone age.
24/ MOPDII	Morocco F	Y	37/1300/36/26	-5.5/-7/-6.5	N		Cranial multiple osteolysis

Table 1: Clinical and radiological features of the 24 families.

Patients 1-11: Patients with Seckel diagnosis - *PCNT* excluded

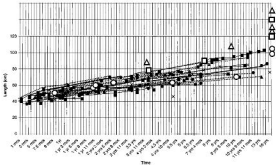
Patients 12-16: Patients with Seckel diagnosis - *PCNT* mutation identified

Patients 17-24: Patients with MOPDII diagnosis - *PCNT* mutation identified

CNS: central nervous system, CS : consanguinity, F: female, FT: full-term pregnancy, HC : head circumference, M: male, MOPDII: Patient with microcephalic osteodysplastic primordial dwarfism type II syndrome N: no, NA: non available, SCKL: Patient with Seckel syndrome, VSD: ventricular septal defect, WOG: week of gestation, Y: presence

Table 2: Mutations identified in our series. (#) This mutation was previously identified by Rauch in a MOPDII patient with the same Turkish ethnic background

Family	Diagnosis	Identified mutation	Position	Status	Protein
12	SCKL	c.1753C>T	Exon 11	homozygous	p.Arg585X
13	SCKL	c.3840G>C	Exon 19	homozygous	p.Gln1280His Splicesite : Pro1204Glyfs*11
14	SCKL	c.6176_6189delGTCA CTGCCGAAG	Exon 30	homozygous	p.Gln2060Argfs*48
15	SCKL	c.3271_3272delTT	Exon 16	homozygous	p.Leu1091Valfs*101
16	SCKL	c.5266dupA	Exon 28	homozygous	p.Met1756Asnfs*53
17	MOPDII	c.3109G>T	Exon 15	homozygous	p.Glu1037X (#)
18	MOPDII	c.9099+2T>C	Intron 40	homozygous	Splicesite
19	MOPDII	c.6316_6325delGTTT GAGAGCA	Exon 30	homozygous	p.Leu2106Alafs*18
20	MOPDII	c.2326_2327delGA	Exon 14	homozygous	p.Glu776Lysfs*3
21	MOPDII	c.3608-2A>G	Intron 18	homozygous	Splicesite
22	MOPDII	c.5578G>T	Exon 28	heterozygous	p.Glu1860X
23	MOPDII	c.7338C>A	Exon 34	homozygous	p.Cys2446X
24	MOPDII	c.3382C>G	Exon 17	homozygous	p.Gln1128X



- Diagnosis of SCKL and PCNT mutation
- Diagnosis of MOPC and PCNT mutation
- △ Diagnosis of SCKL and PCNT excluded

- ▲ Male Article Case
- Male Patient
- Female Article Case
- Female Patient

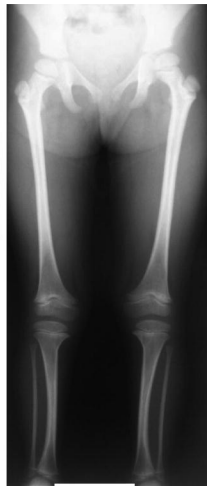
Patients clinically diagnosed as SCKL



Patients clinically diagnosed as MOPDII



Patients clinically
diagnosed as SCKL



5 ½ years



2 years

19 years

Patients clinically
diagnosed as MOPDII

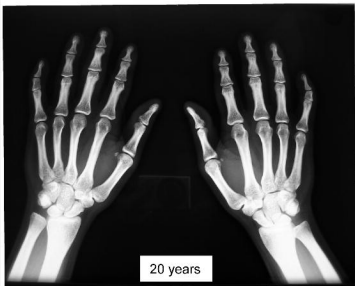


5 years



20 years

Patients clinically diagnosed
as SCKL



Patients clinically diagnosed as
MOPDII

